

PRETESTIS AUSTRALIANUS GEN. ET SP. NOV. (DIGENEA:
PARAMPHISTOMATIDAE) FROM AUSTRALIAN FISH,
AND A CLOSELY RELATED CERCARIA, CERCARIA
ACETABULAPAPILLOSA SP. NOV., WITH NOTES
ON THE LIFE HISTORY

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ABSTRACT

Pretestis australianus gen. et sp. nov., from four Australian freshwater fish, and *Cercaria acetabulopapillosa* sp. nov., from the prosobranch mollusc *Plotiopsis tetrica* Conrad, are described. The cercaria resembles *Pretestis australianus* very closely, but differs in having a papilla at the base of the acetabulum. It is considered possible that examination of fresh material may reveal this structure in *P. australianus*. The cercaria encysts in the open. The redia, cyst and metacercaria are described.

RESUMEN

Se hace la descripción de *Pretestis australianus* gen. y sp. nov., de cuatro peces dulceacuicolas australianos, y de *Cercaria acetabulopapillosa* sp. nov., del molusco prosobranquio *Plotiopsis tetrica* Conrad. La cercaria se asemeja muchísimo a *Pretestis australianus*, aunque difiere por tener una papila en la base del acetábulo. Se considera posible que el examen de material fresco pueda revelar esta estructura en *P. australianus*. La cercaria se enquistó en el medio ambiente. Son descritos la redia, el quiste y la metacercaria.

In 1964, one of us (H. W. M.) collected three specimens of a paramphistome, belonging to a new genus, from the rectum of the silver perch, *Bidyanus bidyanus* (Mitchell) at Goondiwindi, Queensland. Subsequently, when he visited Adelaide, these were compared with a paramphistome cercaria from *Plotiopsis tetrica* Conrad which had been found from time to time in University of Adelaide studies. The cercaria has a well-developed reproductive system; in the details of this, and in other features, it resembles the trematode from *B. bidyanus* so closely that it seems likely that

it is the larval form of this species. Because of the presence of a rounded papilla in the floor of the acetabulum in the cercaria, and the apparent absence of this in the adult trematode, we cannot be certain that the cercaria and adult belong to the same species, so we have described the cercaria as a separate species.

The trematode is also recorded from South Australia, from *B. bidyanus* and from two other fish, *Percalates colonorum* (Gunther) and *Acanthopagrus butcheri* (Munro), and from Queensland from *Melanotaenia fluviatilis* (Castel-

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nau). Most of the specimens were not fully mature.

The description is based on stained mounts of two specimens from *B. bidyanus* from Queensland (the third being somewhat twisted, and lacking the aceta-

bulum). These specimens were killed under coverslip pressure in formol acetic alcohol. Measurements of these and of the specimens from other hosts are given in Table 1. Unless otherwise stated, all measurements are given in microns.

GENERIC DIAGNOSIS

Paramphistomatidae. Acetabulum large, simple, ventro-terminal. Mouth terminal; pharynx with well-developed diverticula; oesophageal bulb with fine concentric muscles; caeca wide, terminating short of acetabular region. Genital pore on left, anterior to anterior testis. Testes round, diagonal, contiguous; anterior one largely extracaecal, on left side, posterior one intercaecal. Cirrus sac present. Ovary near posterior border of posterior testis, to left. Vitelline follicles quite large, in post-testicular region.

Parasites of freshwater and coastal fish.

Pretestis australianus gen. et sp. nov.

(Figs. 1, 2)

Type Host and Locality: *Bidyanus bidyanus* (Mitchell), from the Macintyre River, Goondiwindi, Queensland. (Two fish examined; one infected).

Other Locality: Lower River Murray, South Australia. (Twelve fish examined, one infected).

Type Specimens Deposited. Holotype: South Australian Museum No. E864. Paratype: U.S. National Museum, Helminth. Coll. No. 71927.

Other Hosts: *Melanotaenia fluviatilis* (Castelnau), Goondiwindi, Queensland. (Four specimens from Lower River Murray, South Australia, were uninfected). *Percalates colonorum* (Günther), Lower River Murray. (Seventeen fish examined, two infected). *Acanthopagrus butcheri* (Munro), Lower River Murray. (Two

fish examined, one infected). (Forty-five *A. butcheri* from South Australian coastal waters and nine from Nicholson River, Victoria, were uninfected).

All specimens were found in the digestive tracts of the hosts.

Description. Body flattened, with rounded ends, somewhat narrower anteriorly, greatest width in posterior half. Surface of body pigmented, particularly anteriorly in region of cercarial eyespots. (One eyespot [60 by 37] still intact in paratype). Mouth terminal or ventro-terminal. Acetabulum simple, ventro-terminal. Pharynx* with well-developed diverticula; pharyngeal sphincters not seen. Oesophagus not visible; oesophageal bulb with fine concentric muscles; caeca 68 wide, terminating 1/3 to 2/5 body length from posterior end.

Testes rounded, diagonal, almost contiguous; anterior one largely extracaecal, on left side; posterior testis intercaecal. Seminal vesicle coiled, commencing near anterior margin of posterior testis, passing forward to right of anterior testis; cirrus sac 90 by 47 (holotype), 90 by 60 (paratype). Genital pore 13 by 26, on left, immediately anterior to anterior testis.

Ovary round or elongated (84 by 53 in holotype, 79 by 79 in paratype), level with or posterior to posterior testis; slightly left of midline in holotype, ventrally to left caecum in paratype. No eggs¹ in uterus, but sperm present in

* Näsmark's (1937) terminology is used.

¹ Two eggs in a specimen from *Percalates colonorum* measure 123 by 59 and 129 by 65.

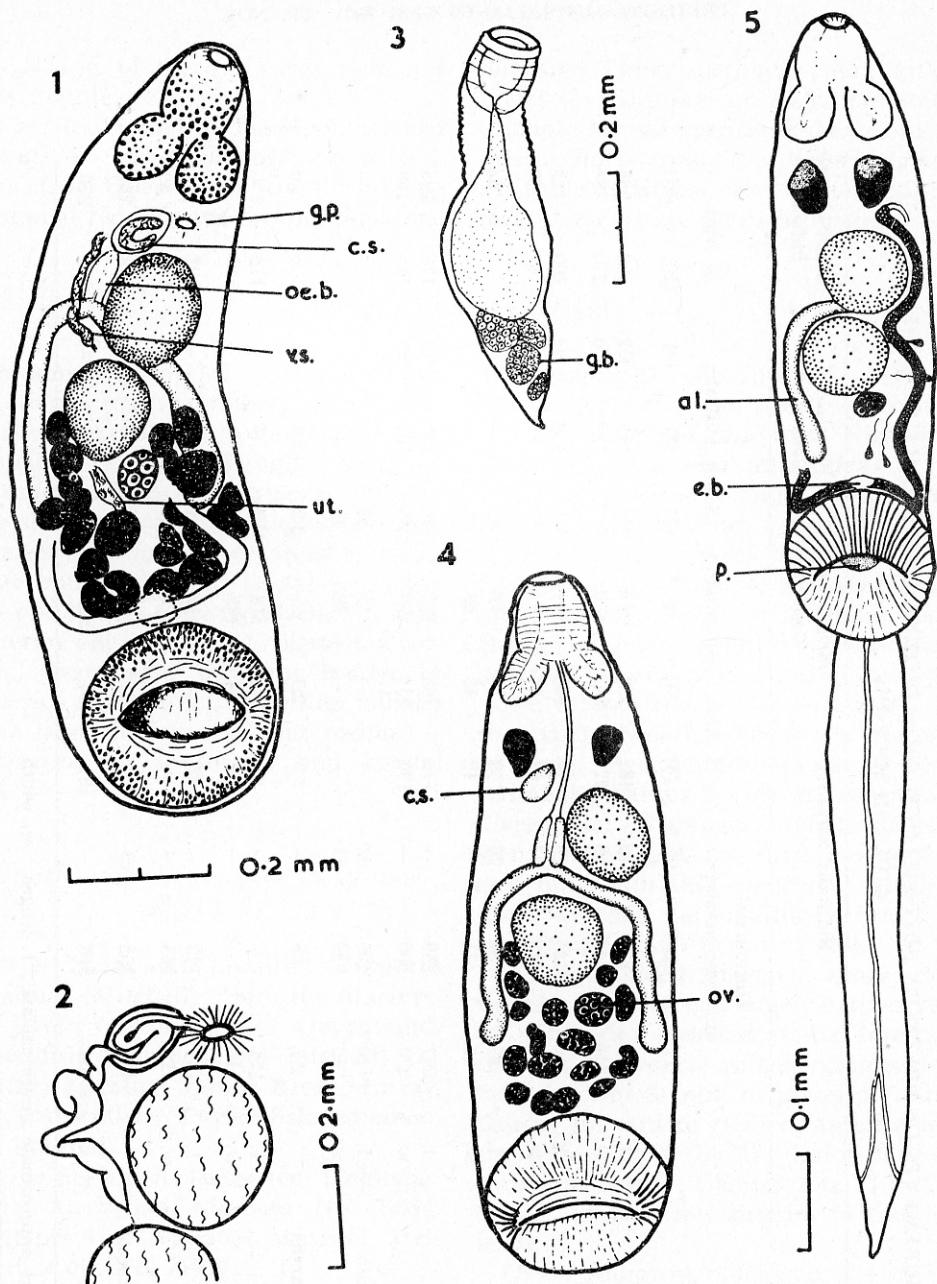
TABLE 1

MEASUREMENTS OF *PRETESTIS AUSTRALIANUS* FROM DIFFERENT HOSTS. ALL MEASUREMENTS GIVEN IN MICRONS

Host		<i>Bidyanus bidyanus</i> .			<i>Melanotaenia fluvialis</i>			<i>Percalates colonorum</i>			<i>Acanthopagrus butcheri</i>	
		Queensland			S. Aust.			South Australia			S. Aust.	
		Holotype paratype										
		(1) *	(2) *	(3) *	(4)	(5)	(6) *	(7)	(8)	(9)	(10)	(11)
Body	l.	1054	918	782	482	553	918	764	917	952	529	588
	w.	340	408	238	223	247	170	212	188	170	270	188
Oral sucker	l.	116	145	118	79	105	97	110	118	92	100	113
	w.	100	110	97	63	87	74	74	79	—	97	87
Oral pouch	l.	87	84	76	50	50	79	68	—	—	—	84
	w.	66	71	—	42	45	63	59	—	—	—	58
Acetabulum	l.	247	259	—	176	165	223	176	210	213	176	208
	w.	270	306	—	190	235	200	235	192	195	188	168
Oesophageal bulb.	l.	"110"	"105"	82	—	—	55	53	—	—	—	—
	w.	"32"	"39"	53	—	—	42	45	—	—	—	—
W. acet./w. pharynx		2.7	2.8	—	3.0	2.7	2.7	3.2	2.4	—	2.0	2.0
Ant. testis.	l.	174	—	—	68	84	118	110	—	—	116	—
	w.	142	—	—	66	84	105	97	—	—	110	—
Post. testis.	l.	147	179	—	60	—	116	113	—	—	97	74
	w.	131	163	—	66	—	92	82	—	—	113	89
Ant. endbifurocaecal		423	353	365	203	218	318	282	—	—	179	250
Caeca-post. end		400	318	—	181	197	329	210	—	—	223	276

" " Indicates approximate measurements. l, length; w, width.

* Fixed under coverglass pressure.



Figures 1, 2. *Pretestis australianus* gen. nov., sp. nov. 1. Holotype, ventral view. 2. Detail of male reproductive system.

Figures 3, 4, 5. *Cercaria acetabulopapillosa* sp. nov. 3. Redia, whole mount. 4. Metacercaria (S. A. Museum, No. E869) 5. Cercaria (S. A. Museum No. E867, with some of the details from other slides, and from living specimens). All drawings were made with the aid of a camera lucida.

Figures 1 and 4 to same scale. Key to lettering. *al.*, alimentary caecum; *c.s.*, cirrus sac; *e.b.*, excretory bladder; *g.b.*, germ ball; *g.p.*, genital pore; *oe.b.*, oesophageal bulb; *ov.*, ovary; *p.*, acetabular papilla; *ut.*, uterus; *vs.*, vesicula seminalis.

some post-testicular coils. Vitellaria approximately thirty in holotype, nineteen in paratype; quite large lobes, rounded or slightly elongated, mostly confined to post-testicular region. No vitelline ducts or reservoir, no ootype or associated structures, evident.

Excretory bladder small, rounded, immediately anterior to acetabulum; wide arms curving to sides of body, then mediad.

Discussion. Twenty or more genera of paramphistomes have been described from freshwater fish. Of these, only *Sandonia* McClelland, 1957 and *Basidiodiscus* Fischthal and Kuntz, 1959 (with one species each) have the same arrangements of the testes diagonal, with one testis extracaecal and one intercaecal. In the young stage of *Sandonia sudanensis* McClelland the testes are intercaecal, but as the worms mature "the testes are displaced anteriorly or laterally so that normally at least one overlaps the intestine or even lies almost completely outside the arc of the intestinal caeca." As our specimens of *P. australianus* are not egg-bearing (with the exception of one specimen from *Percalates colonorum*, which had two eggs) they can be compared with the young stages of *S. sudanensis*. In all of our specimens the anterior testis is largely extracaecal.

Pretestis is further distinguished from *Sandonia*, and from *Basidiodiscus*, in the structure of the acetabulum. In *P. australianus* the acetabulum is large but simple, with the aperture oval, the long axis being across the width of the body. In *S. sudanensis* the aperture of the acetabulum is a tri-radiate slit in young and contracted specimens, triangular or roughly oval in older specimens. From the figures, the long axis of the aperture appears to be in the vertical plane. McClelland noted that around the aperture is a thickened area which appears in sections as a dense pad of tissue much

thicker at the anterior end of the aperture than posteriorly. Fischthal and Kuntz (1958) described the acetabulum as a "muscular, puckerable cup". In *Basidiodiscus ectorchis* Fischthal and Kuntz the acetabulum is terminal and in the form of a pedestal-like appendage, much wider than the base of the body, with twelve prominent papilliform projections on its bottom.

There are other differences between *Pretestis* and the other two genera; such as overall size, the separation of the testes and the posterior position of the ovary in *Sandonia* and *Basidiodiscus*. These may be attributable to the difference in age, and the presence of many eggs in the uterus, in the two latter genera. A further comparison will have to await study of fully mature, egg-bearing specimens of *P. australianus*.

The only paramphistomes previously recorded from Australian fish are *Chononymyzus tasmaniae* Manter and Crowcroft, 1950, from a marine fish, *Dactylosargus arctidens*, and *Bancroftrema neoceratodi* Angel, 1966 from the Australian lungfish, *Neoceratodus forsteri*. In both of these forms the testes are symmetrical and intercaecal.

Cercaria acetabulopapillosa sp. nov.
(Figs. 3-5)

Material deposited in South Australian Museum. Slides (whole mounts): rediae Nos. E865, E866; cercariae, Nos. E867, E868; metacercariae, Nos. E869, E870. Spirit material: cercariae, No. E871; gills of *Plotiopsis tetrica* with rediae and cercariae, No. E872; two *Plotiopsis tetrica* with rediae and free cercariae, No. E873; cysts, No. E874.

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some post-testicular coils. Vitellaria approximately thirty in holotype, nineteen in paratype; quite large lobes, rounded or slightly elongated, mostly confined to post-testicular region. No vitelline ducts or reservoir, no ootype or associated structures, evident.

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snails have been collected at several places on the lower River Murray; the greatest number of infections (21) was from Morgan.

The cercaria is large and obvious. Not many cercariae are emitted daily by infected snails, and the number diminishes quickly, possibly due to malnutrition of the host under laboratory conditions. Although one snail under observation ceased to give off cercariae within 10 days, another gave them intermittently for 130 days.

REDIA

The digestive gland of the snail is quite free of rediae, which occur in the wall of the mantle. They sometimes seem to accumulate near the wall of the intestine in the mantle area. Free cercariae are present in abundance in some infected snails. Some are still within the mantle, but others must shelter within the shell, judging by the number which are found swimming in a dish when the snail shell is broken and removed, even though the snail tissues remain intact.

Formalinised rediae averaged about 800 by 190; the largest seen was 884 long. The most noticeable feature of the redia is the wide open mouth, up to 58 across, which gives the impression of a ring of tissue around the anterior end. The posterior end narrows to a sharp tip. The pharynx is thick-walled and obvious; in a specimen 765 long, it measured 153 by 118, the walls being 24 in thickness. Following the pharynx is an oesophagus, straight or coiled, depending on the contraction of the redia. The intestine, from 59 to 153 wide, extends to between half and three-quarters of the body length. Its contents are finely granular. There appears to be no more than one developed cercaria in each redia, though several developing ones, tail-less, are generally also present.

CERCARIA

Cercariae for measurement were fixed in two ways. In general, an equal volume of boiling 10% formalin was added to water containing the cercariae. Ten of these measured: body, 323-576 (av. 396) by 212-288 (240); tail, 753-940 (847) by 65-76 (71). One collection was fixed by pipetting cercariae into 5% formalin just off boiling point. The bodies of these were better extended than those fixed by the former method, and the body and tail were fixed more or less in a straight line; body, 612-717 (670) by 162-176 (168); tail, 623-676 (650) by 65-76 (71).

The description is based on living specimens and temporary and permanent mounts. Measurements of organs, etc., are averages taken from the holotype and one paratype (well extended specimens, mounted in balsam, 635 by 159 and 647 by 141 respectively).

Description. Mouth terminal; pharynx 64 by 62; with thick-walled pouches, 51 by 39; oesophagus not seen; oesophageal bulb near dorsal surface; bifurcation of caeca 235 from anterior end of body; caeca 32 wide, ending 220 from posterior end. Acetabulum ventro-terminal, 140 by 140, strongly muscular, with rounded elevation, 32 across, arising from floor of cavity. Tail arising dorsally. Heavily pigmented eyespots 50 by 26, 29 apart, near dorsal surface, close behind level of pharyngeal pouches, cone-shaped, with antero-dorsal part hollowed as if to contain lens.

Anlagen of reproductive system well-developed. Genital pore 12 by 21, anterior to anterior testis, or median; leading into small genital atrium, about 18 deep. Cirrus complex present. Testes 87 by 74, contiguous, generally diagonal, with anterior testis anterior to, and overlapping, left caecum; some specimens have ante-

rior testis median or to right but always largely extracaecal. Ovary round, 43 across, immediately posterior to posterior testis. Vitellaria consisting of about 24 rounded lobes, 18 across; occupying post-testicular area and extending laterally to mid-level of posterior testis.

Subcuticular area of body filled with elongated, brownish-yellow rods, of cystogenous material, arranged in groups of ten or so; rods generally in pairs, with members of pair end to end and pairs in parallel. Adjoining groups set at different angles to each other. Individual rods measure 10-13 by 2.5.

Thick concentration of small granules of pigment immediately below cuticle, with larger aggregations of granules in tissues.

Excretory bladder immediately anterior to acetabulum. Arms of bladder filled with small granules (three or four across diameter of tube); arms hugging anterior border of acetabulum, then winding to level of eyespots; recurrent tubes without granules, reaching nearly to acetabulum.

The presence of the rods of cystogenous material and the pigmentation of the body prevents elucidation of the details of the excretory system. Only four flame cells have been seen-lying between the anterior border of the posterior testis and the acetabulum. On each side of the acetabulum is a number of twisted tubes; several of the branches end in two fine tubes, but we have been unable to see any flame cells here. The tubes may be part of the lymphatic system, though Willey (1930) thought that the lymph system was probably not present in the cercarial stage of amphistomes.

The excretory tube in the tail bifurcates about 80 from the tip, the two branches terminating at the sides of the tail, though apparently without external opening.

CYST

In February, 1964, after infected snails had been isolated in 3 by 1 inch tubes for some days, cysts were observed on the filamentous alga (family Cladophoraceae) which commonly grows on the shells of *Plotiopsis tetrica*. In all, 23 cysts were recovered from two infected snails kept under observation as long as they were emitting cercariae. In May, 1967, one infected snail yielded 8 cysts; but in April, 1968, no cysts formed on the alga on a snail which was producing several cercariae daily. The cysts show up clearly as small black dots. The fact that they had not been observed prior to 1964, together with the erratic occurrences after 1964, suggests that the alga is not the only site used for encystment.*

Cysts have never been observed on the sides of the isolation tubes, nor on the shells of infected snails. After boiling formalin had been added to water in which cercariae were swimming, a small number of cysts was found among the cercariae. These may have encysted freely, but some of them had the appearance of having been attached, with extensions at one edge of the cyst wall.

The cysts are roughly spherical in shape, but are often flattened along one margin, where they have been attached. Ten cysts measured before fixation averaged 259 by 242.

Encystation was occasionally observed under a coverslip. This occurred if the cercariae were allowed enough fluid in which to move around freely. It rounded up quickly and a clear mucus-like exudate enclosed it. The rod-shaped granules were extruded from the surface of the cercaria and became caught up in the mucus to form a cyst wall.

* It is of interest that filamentous alga is found commonly in the stomachs of *Bidyanus bidyanus* and *Acanthopagrus butcheri*, in both of which fish *Pretestis australianus* has been found.

METACERCARIA

Seven encysted metacercariae, flattened under a coverslip and fixed in formalin, measured 690 by 238 to 1045 by 290. They were afterwards stained in Van Cleave's combination stain and mounted in balsam.

Description. Body heavily pigmented, eyespots still present. Pharynx, pharyngeal pouches, oesophageal bulb and caeca as in cercaria; oesophagus a narrow straight tube. Reproductive system as in cercaria, but ovary and vitellaria better defined; anterior testis on right side in four of six specimens.

LIFE HISTORY EXPERIMENTS

Two experiments to obtain the adult of *C. acetabulopapillosa* gave negative results. In February, 1964, a rat was fed cheese with 23 cysts; it was killed in May. In May, 1967, an attempt was made to feed a few cysts and some metacercariae, to a carp but it is not certain that the carp actually swallowed the trematodes.

Discussion. *Cercaria acetabulopapillosa* resembles *Pretestis australianus* very closely, and may even be the larval stage of this trematode. The presence of an acetabular papilla in the cercaria and its apparent absence in the adult makes it necessary to give the cercaria a distinctive name. However, the papilla was not noted when living cercariae and metacercariae were examined under a coverslip; it was not until formalinised cercariae were cleared that this feature became obvious. The surface of the elevation seems to be finely mammillated; it does not suggest a sucker in any way, and there is no obvious muscular structure. It seems likely that in living cercariae and metacercariae, which were examined under quite heavy coverslip pressure, the

elevation was flattened down and rendered inconspicuous. The same may have happened with the specimens of *P. australianus*. The acetabulum as seen in mounted metacercariae resembles that of *P. australianus* very closely. In S. A. Museum No. E870 it would appear identical with the acetabulum of the adult trematode. Verification of *C. acetabulopapillosa* as the larval stage of this trematode will have to await further life-history studies, or at least the examination of specimens of *P. australianus* fixed in the same way as were the cercariae — in hot formalin, without previous flattening.

The fact that the anterior testis may be median or on the right, as well as on the left side in *C. acetabulopapillosa* agrees with the observations of Fischthal and Kuntz (1959), for *Sandonia sudanensis* and *Basidioliscus ectorchis*, that the anterior testis may be on the left or the right side. This variation cannot be regarded as constituting a difference between *C. acetabulopapillosa* and *Pretestis australianus*, in view of the small number of the latter which have been examined from each host. (It is possible that the position of the ovary and of the genital pore may also be variable).

According to Yamaguti (1958), the only genera of the *Paramphistomatidae* in which the acetabulum has a central prominence are some members of the *Diplodiscinae*. Of these, *Opisthodiscus* and *Pseudopisthodiscus* have "a suckerlike central navel"; *Diplodiscus* has a central papilla; and *Megalodiscus* may or may not have a central papilla. All of these genera occur in amphibians. (*Diplodiscus cornu* (Dies., 1839) Daday, 1907, from a fish (*Doras* sp.), was transferred by Manter (1962) to a new genus, *Pseudodiplodiscus*, one of the characters of which is "acetabulum without central papilla".)

If *C. acetabulopapillosa* proves to be the larva of *Pretestis australianus*, this will be the first fish trematode so far

recorded with a central papilla in the acetabulum.

No life history has yet been recorded for a paramphistome from a fish.

The snail host of *Cercaria acetabulopapillosa*, *Plotiopsis tetrica*, is a prosobranch gastropod belonging to the Thiariidae.* Although a good many paramphistome cercariae have been described, nearly all have developed in opisthobranch molluscs. We have been able to find only two apparently valid references to a prosobranch host for this family.² Loos (1896) [quoted by Fairley and

Bahr (?1919) and Yamaguti (1958)] reported a paramphistome cercaria (which he referred to *Gastrodiscus aegyptiacus* [Cobbold]) from *Cleopatra bulimoides*. Fairley and Bahr (?1919) figured a paramphistome cercaria; in the description of the figure they noted that "under this heading" were included cercariae probably of three species described by Loos (1896). They were found most commonly in *Bullinus contortus* but were occasionally seen in *Planorbis boissyi*, *Limnaea caillaudi* and *Cleopatra bulimoides*. *C. bulimoides* is a prosobranch which also belongs to the Thiariidae. It is worthy of mention that Peter (1960) pointed out that Loos' conclusion that the cercaria from this host was the larva of *Gastrodiscus aegyptiacus* was based on the structural similarity between the cercaria and the adult, and was not supported by any experimental evidence.

* Wenz's (1938) classification is used.

² Although Yamaguti (1958) quoted Cary (1909) as saying that the cercaria of *Megalodiscus temperatus* (Stafford) developed in *Goniobasis virginica* (which is placed in the Thiariidae), Cort (1915) showed that Cary had used, in his experiments, two entirely different species of cercariae, neither of which could have developed into a paramphistome. Krull and Price (1932) stated that *Helisoma trivolvis* was the only snail which had been found infected with *Diplodiscus temperatus* Stafford.

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